*HRD*ing to the Slaughter: The Destruction of a Translocon-Clogging Protein

An Honors Thesis (HONR 499)

By

Sam Coffey

Thesis Advisor
Eric Rubenstein

Ball State University
Muncie, Indiana

May, 2014

Expected Date of Graduation

May, 2014

Abstract

Proper protein synthesis and degradation is essential to cell health. A number of human diseases and pathologies can be attributed to improper protein creation and destruction. Historically, the model organism *Saccharomyces cerevisiae* has been used to elucidate the biochemical pathways involved in protein degradation. In cells, proteins are degraded via the ubiquitin-proteasome system at several locations, including the cytosol, nucleus, mitochondria and endoplasmic reticulum. Recently, a novel form of endoplasmic reticulum-associated protein degradation (ERAD) was discovered. This novel degradation system was found to be related to proteins clogging an essential endoplasmic reticulum channel, known as the translocon, and thus termed ERAD-T. We attempted to develop a yeast growth-based assay in order to facilitate the characterization and identification of the genetic requirements of ERAD-T. Additionally, we endeavored to isolate a model ERAD-T substrate through immunoprecipitation so that the post-translation modifications possibly involved with ERAD-T can be determined.

Acknowledgements

I would like to express the utmost gratitude to my thesis advisor, mentor, and lab supervisor, Dr. Eric Rubenstein. His patient nature, passion for science, exceptional teaching ability, and general willingness to go above and beyond to ensure the success of his students has contributed immensely to my positive undergraduate experience. Without his extensive knowledge of the inner workings of yeast and the techniques used to determine those workings, this research would not have been possible.

I would also like to thank Dr. Susan McDowell for her teaching contributions to my scientific technique and the many positive influences she provided.

Furthermore, I would like to express my gratitude to the 2013-2014 Rubenstein lab members including Justin Crowder, Sheldon Watts, Eric Fults, and Ian Tesch for contributing to the many lab solutions used through the course of this thesis research, taking the time to teach me new experimental techniques, and for always making lab enjoyable and exciting.

I would to express gratitude to my parents whose constant support and general life advice has been instrumental not only in the creation of this thesis but also my college experience as a whole.

Finally, I would like to acknowledge that this research would not have been possible without funding from Ball State University and the Ball State chapter of Sigma Xi.